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Prevalence of high risk human papillomavirus (HPV) infection and abnormal cervical cytology and knowledge about HPV vaccine in Eastern Turkey

H.G. Sahin[1], A. Kolusari[1], H. Guducuoglu[2]

[1] Department of Obstetrics and Gynaecology, Faculty of Medicine, Yuzuncu Yil University, Van
[2] Department of Microbiology and Clinical Microbiology, Faculty of Medicine, Yuzuncu Yil University, Van (Turkey)

Summary

Purpose of investigation: The aim of the present study was to gather data on the high risk human papillomavirus (HPV) frequency and the distribution of HPV types in Eastern Turkey in relation to cervical cytology and to assess the knowledge about cervical cancer, HPV, and vaccination. *Materials and Methods:* The study population included 1,000 women aged between 20-65 years who attended the outpatients clinics of Department of Obstetrics and Gynecology of the Medical School of Yuzuncu Yıl University Hospital. *Results:* The overall prevalence of high risk HPV-DNA was 2.8 %. Abnormal cytology was observed in 12.9% of the cases. Abnormal cytology consisted of cervical cancer: 0.1%, LSIL: 1.6%, HSIL: 0.5%, ASC-H: 2.1%, atypical glandular cells: 0.4%, and ASCUS: 8.3 %. *Conclusion:* The prevalence of HPV was low in Eastern Turkey and the most common HPV types are similar to the literature. However, the prevalence of HPV infection is a growing problem worldwide and the awareness of the women in the region is limited.

Key words: Human papillomavirus; Cervical cytological samples; Uterine cervical neoplasm; Turkey.

Introduction

Cervical cancer is the fourth most common cancer effecting women worldwide after breast, colorectal, and lung cancer. With 528,000 new cases every year, it is the fourth most common cause of cancer deaths. It is reported that it caused 266,000 deaths in women in 2012 worldwide and 70% of these deaths occurred in developing countries [1]. Like the prior studies, Globocan 2012 highlights that priority should be given to cancer prevention and control measures for breast and cervical cancers globally [1, 2].

Cervical cancer is widely accepted to be related to oncogenic human papillomavirus (HPV) infection since the hypothesis published in 1976 [3]. HPV is a common viral sexually transmitted disease and it is reported that more than 50% of women become infected by the age of 20-30 years [4]. More than 150 HPV genotypes have been described [5]. HPV type 16 and/or 18 are considered as the causative agents in 70 % of cervical cancers and the next six most common types were reported to be HPV 45, 31, 33, 52, 58 and 35, accounting for the remaining 20% of cervical cancer cases worldwide [6, 7]

Screening programs in developed countries have reduced mortality and morbidity from cervical cancer. However, it remains to be an important public health problem worldwide [1].

Since 2007, prophylactic vaccines against HPV are available, targeting the capsid protein of the two most frequent carcinogenic genotypes: HPV 16 and 18. Although HPV 16 and 18 are the most prevalent types involved in cervical cancer development worldwide, other high risk HPV types may show relative importance among different populations. Therefore reliable baseline estimates of the distribution of type of cervical HPV infection are essential to assess the potential benefit of the HPV vaccination at the national and even regional level.

Data about distribution of HPV types in Eastern Turkey is lacking.The aim of the present study was to gather reliable data on the high risk HPV frequency and the distribution of HPV types in relation to cervical cytology, the knowledge of women about HPV, cervical cancer, and attitude of women against HPV vaccine in Eastern Turkey.

Materials and Methods

The study population included 1,000 consecutive women, aged between 20-65 years who attended the outpatients clinics of the Medical School of Yuzuncu Yıl University, Department of Obstetrics and Gynecology for regular gynecological control between December 2012 and June 2013. Hysterectomized women and women who had cervical cancer and known premalign lesion were excluded from the study. The study was approved by the Ethical Committee and informed consent was obtained from each participant included in the study.

A conventional Papanicolaou (Pap) smear test and a second sam-

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Table 1. — *Prevalence of HPV-DNA types.*

HPV Type	N.	%
16	3	10.7
18	5	17.9
67	3	10.7
52	1	3.6
11/16 (mixed type)	4	14.3
6/16 (mixed type)	3	10.7
39	2	7.1
45	2	7.1
35	2	7.1
70	1	3.6
33/56 (mixed type)	1	3.6
83	1	3.6
Total	28	100.0

Table 2. — *Prevalence of abnormal cytology.*

	N.	(%)
Normal	871	87.1
Invasive cervical cancer	1	0.1
LSIL	16	1.6
HSIL	5	0.5
ASC-H	20	2.0
Atypical glandular cells	4	0.4
ASCUS	83	8.3
Total	1000	100.0

Table 3. — *Presence of high risk HPV-DNA among abnormal and normal cytology.*

	HPV(-)	HPV(+) N (%)	Total
Normal	857	15 (1.7)	871
Cervical cancer	0	1 (100)	1
LSIL	13	3 (18.7)	16
HSIL	2	3 (60)	5
ASC-H	19	1 (5)	20
Atypical glandular cells	3	1 (25)	4
ASCUS	79	4 (4.8)	83

Table 4. — *Presence of high risk HPV-DNA types among abnormal and normal cytology.*

	Mixed HPV 16/11, 16/6, 33/56)	HPV 16, 18	HPV other	Total
Normal	4	6	5	15
Cervical cancer	0	0	1	1
LSIL	1	0	2	3
HSIL	0	2	1	3
ASC-H	0	0	1	1
Atypical glandular cells	0	0	1	1
ASCUS	3	0	1	4

ple was taken with cervical brush and preserved in preservative fluid (liquid based Pap test) for HPV investigation. The samples were sent to the laboratory of the Department of Microbiology of Yuzuncu Yıl University. The smears were sent to the Department on Pathology of Yuzuncu Yıl University. Bethesda System's terminology was used to classify the cervical smear results. Both studies: cytology and HPV testing were performed blinded to the results of the other one. A questionnaire from that was adapted to Turkish population was used to evaluate the awareness of the participants related to relation between HPV and cervix cancer and acceptance of HPV vaccine in women in Eastern Turkey [8]. In order to encourage honest answers, the anonymity and confidentiality of the questionnaire were emphasized at the start of the interview. The study population was interviewed with a pretested questionnaire that consisted of 14 questions. Information was collected on socio-demographic characteristics, knowledge of women about HPV and cervical cancer, and attitude of women against HPV vaccine.

Specimen processing for HPV-DNA testing

HPV extraction was performed with HPV high risk screen FEP kit according to the manufacturer's instructions. After preparation of the samples by using real time polymerase chain reaction (PCR) amplification and multi-channel rotor type fluorescence detector, HPV types were detected. PCR-mix-1 FEP HPV-1 detects HPV types 31, 35, 39, 59, and 16, and PCR-mix-1 FEP HPV-2 detects HPV types 18, 33, 45, 52, 58, and 67.These types are considered to have high neoplastic transformation activity and are responsible for more than 92% of cases of cervical intraepithelial lesions and invasive carcinoma.

HPV genotyping

HPV sign Q24 complete kit, "Rotor-Gene", and "PyroMark Q24" assay systems were used for HPV genotyping. HPV positive samples were further analyzed by pyrosequencing of DNA.

The data were recorded for each person into a computer database. For statistical analysis, SPSS 17.0 software was used. Logistic regression analysis was performed to determine the variables that had significant correlation with HPV and abnormal cervical cytology. Proportion test was used to compare some variables in terms of rates. A value of $p < 0.05$ was accepted as statistically significant.

Results

A total of 1,000 women who applied for regular gynecological control were included in the study.The mean age was 42.1 years, 13.9% of the patients were nulliparous and the remaining were 8.5% primiparous, 39.3% multiparous, 29.9% grand multiparous, 8.5% great grand multiparous, and 14.4% of the patients were in the postmenopausal period. The rate of illiterate women was 70.9%, 21.5% graduated from primary school 4.5% from high school, 3% from university, and 85% of the respondents did not smoke.

The overall prevalence of high risk HPV-DNA was 2.8%. The most common types were HPV-16: 35.7% (single infection: HPV-16: 10.7%, mixed infection HPV-16/11: 14.3%, HPV-16/6: 10.7%) followed by HPV-18: 17.9%, (Table 1).

Using Bethesda System's terminology, normal cytology was observed in 87.1% of the cases. Abnormal cytology consisted of atypical squamous cell of undetermined significance (ASCUS) 8.3%, low-grade squamous intraepithelial lesions (LSIL) 1.6%, atypical squamous cells can-

not exclude high-grade squamous intraepithelial lesions (ASC-H) 2%, high-grade squamous intraepithelial lesions (HSIL) 0.5%, atypical glandular cells 0.4%, and invasive cervical cancer 0.1% (Table 2). Presence of HPV-DNA among abnormal and normal cytology was 10% and 1.7%, respectively (Table 3). Distribution of cervical cytology with regards to HPV-DNA status in patients with abnormal cytology is presented in Table 4. Eight women (28.5%) had mixed infection and the remaining were infected with single infection.

No significant correlation was found between abnormal cytology, HPV-DNA seropositivity and age, education, smoking, gravidity, and parity. The knowledge of women about HPV, cervical cancer, and HPV vaccine was very low: 98.9% the women in the study did not know the relation between HPV and cervical cancer, 98.7% did not know the vaccine, and 76.8% stated that they were not willing to be vaccinated.

Discussion

On the basis of epidemiologic and virological studies, it is well known that some kinds of cancers are related to infections of oncogenic viruses. Cervical carcinoma is one of these cancers and it is accepted that the cause of almost all of the cases are persistant oncogenic HPV infections [9]. With screening programs, oncogenic HPV-DNA can be detected with PCR and the detection of prevalent HPV genotypes in certain areas of the world is important for the HPV vaccination era. Turkey is located between Europe and Asia and information regarding the prevalence of HPV is reported in several studies that mainly give information on the Western regions of Turkey. Data on HPV prevalence of Eastern Turkey is limited.The prevalence among low-risk women reported by previous studies from hospitals located in Western Turkey ranges between 2-6%, which is much lower than the rate reported by Kunter *et al.* and Polat *et al.* of 20% and 25%, respectively [10-12]. The authors stated that the discrepancy may be due to patient profile and the methods used for detection of HPV as in previous studies the method used was hybrid capture. In this study the authors used PCR and they found the prevalence of high risk HPV to be 2.8%. This rate is lower than the rate reported by Kunter *et al.* and Polat *et al.* [10-12]. Polat *et al.* attributed their high prevalence to high rate of abnormal cytology and higher education level of the study population [12]. The profile of the patients in the study reported by Polat *et al.* are different from the low-risk population in other studies; the rate of illiterate women was 70.9%, 85% of the respondents did not smoke, and the rate of abnormal cytology was 12.9% [12].

In the current study the overall high- risk HPV prevalence in women with normal cytology was 1.7%, which was much lower than the reported prevalence 8.7%, 14.3%, and 5.2% for Asia, South America, and Europe, respectively [13]. The reason most likely that the present study only included high-risk HPV types, which is a limitation of the study. The cases who are high-risk HPV positive with concurrently negative cervical cytology should be followed more frequently than the HPV negative cases as they have greater risk of developing abnormal cytology.

The overall abnormal cytology was 12.9% with cervical cancer 0.1%, LSIL 1.6%, HSIL 0.5%, ASC-H 2.1%, atypical glandular cells 0.4%, and ASCUS 8.3% which was similar to study reported by Kunter *et al.* which was 11.7% [11]. However in contrast to the rate 1.8 % reported in a large multicentric study that included more than 140,000 Turkish women [14]. The most prevalent cervical pathology in the present study was ASCUS (8.3%). However only 4.8% of these cases had positive HPV. This could be explained by over-diagnosis of ASCUS or their is another cause which needs evaluation with further studies. Presence of positive high-risk HPV in cases with abnormal cytology were 18.7% in LSIL, 60% in HSIL, 5% in ASC-H, 25% in atypical glandular cells, 4.8% in ASCUS, and there was one cervical cancer with positive HPV.

The most common two types were HPV-16: 35.7% (single infection: HPV-16: 10.7%, mixed infection HPV-16/11: 14.3%, and HPV-16/6: 10.7%), followed by HPV-18: 17.9%, similar to Western regions of Turkey and Western countries and the third most common was HPV 67: 10.7% which is much lower in other regions of Turkey [10-12]. Eight women (28.5%) had mixed infection and the remaining were infected with single infection. The rate of mixed infection is higher than the previous reports from Turkey but similar to the study reported by Berois *et al.* [10-12,15].

In contrast to previous studies, there was no significant correlation between abnormal cytology, HPV-DNA seropositivity and age, education, smoking, gravidity, and parity [10-12]. The knowledge of women about HPV, cervical cancer, and HPV vaccine was very low: 98.9% the women in the study did not know the relation between HPV and cervical cancer, 98.7% did not know about the vaccine, and 76.8% stated that they were not willing to be vaccinated. The knowledge of women about HPV and cervical cancer and HPV vaccine was lower than the results of Tonguc *et al.*, as their study was also conducted in Eastern Turkey [16]. The most important reason for this is probably due to high rate of illiterate women in the present study which was 70.9%.

In conclusion, cervical cancer is an avoidable cancer; vaccination, effective screening, early detection, and treatment significantly will reduce the cervical cancer deaths. The prevalence of high-risk HPV was low in Eastern Turkey; the most common HPV types were 16 and 18, similar to the Western regions and Western countries. However, the prevalence of HPV infection is a growing problem worldwide and the awareness of the women in the region is limited. It is essential to establish strategies to increase

awareness and acceptance with primary care and prevention specially focusing on illiterate women.

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References

[1] Ferlay J., Soerjomataram I., Ervik M., Dikshit R., Eser S., Mathers C., *et al.*: "GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012 v1.0. IARC CancerBase No. 11". Lyon, France: International Agency for Research on Cancer. Available at: http://globocan.iarc.fr

[2] Nour N.M.: "Cervical cancer: a preventable death". *Rev. Obstet. Gynecol.*, 2009, *2*, 240.

[3] The Nobel Prize in Physiology or Medicine 2008 [press release]. Stockholm, Sweden: The Nobel Assembly at Karolinska Institutet; October 6, 2008. Available at: http://nobelprize.org/nobel_prizes/medicine/laureates/2008/press.html

[4] Kjaer S.K., Breugelmans G., Munk C., Junge J., Watson M., Iftner T.: "Population based prevalence, type and age specific distribution of HPV in women before inroduction of an HPV vaccination program in Denmark". *Int. J. Cancer*, 2008, *12*, 1864.

[5] Bernard H.U., Burk R.D., Chen Z., van Doorslaer Kzur Hausen H., de Villers EM.: "Classification of papillomaviruses(PVs)based on 189 PV types and proposal of taxonomic amendments". *Virology*, 2010, *401*, 70.

[6] De Sanjose S., Quint W.G., Alemany L., Geraets D.T., Klaustermeier J.E., Lloveras B., *et al.*: "Retrospective International Survey and HPV Time Trends Study Group. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study". *Lancet. Oncol.*, 2010, *11*, 1048.

[7] Smith J.S., Lindsay L., Hoots B., Keys J., Franceschi S.,Winer R., *et al.*: "Human papillomavirus type distribution in invasive cervical cancer and high grade cervical lesions:A meta analysis update". Int. *J. Cancer*, 2007, *121*, 621.

[8] Ozyer S., Uzunlar O., Ozler S., Kaymak O., Baser E., Gungor T., *et al.*: "Awareness of Turkish female adolescents and young women about HPV and their attitudes towards HPV vaccination". *Asian Pac. J. Cancer Prev.*, 2013, *14*, 4877.

[9] Walboomers J.M., Jacobs M.V., Manos M.M., Bosch F.X., Kummer J.A., Shah K.V., *et al.*: "Human papillomavirus is a necessary cause of invasive cervical cancer worldwide". *J. Pathol.*, 1999, *189*, 12.

[10] Dursun P., Ayhan A., Mutlu L., Çağlar M., Haberal A., Güngör T., *et al.*: "HPV types in Turkey: multicenter hospital based evaluation of 6388 patients in Turkish Gynecologic Oncology Group centers". *Turk. Patoloji. Derg.*, 2013, *26*, 210.

[11] Yuce K., Pinar A., Salman M.C., Alp A., Sayal B., Dogan S., *et al.*: "Detection and genotyping of cervical HPV with simultaneous cervical cytology in Turkish women: a hospital based study". *Arch. Gynecol. Obstet.*, 2012, *286*, 203.

[12] Dursun P., Senger S.S., Arslan H., Kuscu E., Ayhan A.: "Human papillomavirus prevalence and types among Turkish women at a gynecology outpatient unit". *BMC Infect. Dis.*, 2009, *9*, 191.

[13] Clifford G.M., Gallus S., Herrero R., Munoz N., Snijders P.J., Vaccarella S., *et al.*: "Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis". *Lancet*, 2005, *366*, 991.

[14] Turkish Cervical Cancer and Cervical Cytology Research Group: "Prevelance of cervical cytological abnormalities in Turkey". *Int. J. Gynaecol. Obstet.*, 2009, *106*, 206.

[15] Berois N., Heard I., Fortb Z., Alonso R., Sica A., Moerzinger P., *et al.*: "Prevalence of type specific HPV infection in Uruguay". *J. Med. Virol.*, 2014, *86*, 647.

[16] Tonguc E., Gungor T., Var T., Kavak E., Yucel M., Uzunlar O.: "Knowledge about HPV, relation between HPV and cervix cancer and acceptance of HPV vaccine in women in eastern region of Turkey". *J. Ggnecol. Oncol.*, 2013, *24*, 7.

Corresponding Author:
A. KOLUSARI, M.D.
Yuzuncu Yil Universitesi
Tip fakültesi, Zeve kampusu
65200 Van (Turkey)
e-mail: dralikolusari@yahoo.com